

1321 GATTCCAAGG AACACAGTGG TGCCTACCAA GAAAGTCTCAG ATCTTTCTA CAGCTTCTGA
1381 TAATCAACCA ACTGTTACAA TCAAGGTCTA TGAAGGTGAA AGACCCCTGA CAAAAGACAA
1441 TCATCTTCTG GGTACATTG ATCTGACTGG AATTCCCTCCT GCTCCTCGTG GGGTCCCACA
1501 GATTGAAGTC ACCTTTGAGA TAGATGTGAA TGGTATTCTT CGAGTGACAG CTGAAGACAA
1561 GGGTACAGGG AACAAAAATA AGATCACAAT CACCAATGAC CAGAATCGCC TGACACCTGA
1621 AGAAATCGAA AGGATGGTTA ATGATGCTGA GAAAGTTGCT GAGGAAGACA AAAAGCTCAA
1681 GGAGCGCATT GATACTAGAA ATGAGTTGGA AAGCTATGCC TATTCTCTAA AGAACATCAGAT
1741 TGGAGATAAA GAAAAGCTGG GAGGTAAACT TTCCTCTGAA GATAAGGAGA CCATGGAAAA
1801 AGCTGTAGAA GAAAAGATTG AATGGCTGGA AAGCCACCAA GATGCTGACA TTGAAGACTT
1861 CAAAGCTAAG AAGAAGGAAC TGGAAAGAAAT TGTTCAACCA ATTATCAGCA AACTCTATGG
1921 AAGTGCAGGC CCTCCCCCAA CTGGTGAAGA GGATACAGCA GAAAAAGATG AGTTGTAGAC
1981 ACTGATCTGC TAGTGCTGTA ATATTGT

B
A

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 through 4, at the end of the application and renumber the pages of the application accordingly.

REMARKS

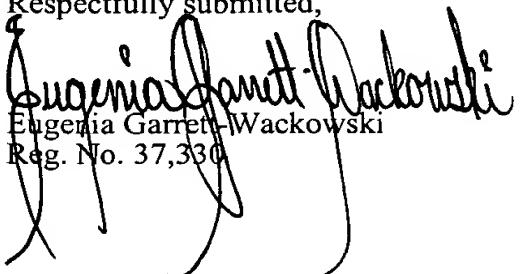
Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-5, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the specification by the amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning on page 38, line 2 has been amended as follows:

To obtain cells stably expressing GRP78/BiP, T24/83 cells were transfected with either the mammalian cell expression vector pcDNA3.1(+) or pcDNA3.1(+) containing the open reading frame of human GRP78/BiP. The latter vector was obtained by amplifying the cDNA encoding the open-reading frame of human GRP78/BiP (approximately 1.95 kb) by reverse transcriptase-PCR using total RNA from primary HUVEC. GRP78/BiP cDNA was generated using SuperScript RNase H reverse transcriptase (Gibco/BRL) and a primer complimentary to a sequence in the 3'-untranslated region of the human GRP78/BiP mRNA transcript (AB10230; 5'-TATTACAGCACTAGCAGATCAGTG-3')(SEQ ID NO:1). For PCR amplification, the forward primer AB10231 (5'-
CTTAAGCTT**GCCACC**ATGAAGCTCTCCCTGGTGGCCGCG-3')(SEQ ID NO:2) contained a Kozak consensus sequence (bold) prior to the initiating ATG and a terminal *Hind*III restriction site (underlined). The reverse primer AB10232 (5'-
AGGCCTCGAGCTACAAC~~T~~CATCTTTCTGCTGT-3')(SEQ ID NO:3) contained a terminal *Xho*I restriction site (underlined) adjacent to the authentic termination codon of the GRP78/BiP cDNA. PCR reactions took place in a final volume of 50 µl containing 2 µl of the RT reaction, 100 ng of primers, 2.5 U *Taq* polymerase (Perkin-Elmer, Mississauga, ON) in a buffer consisting of 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.8) and 0.5 mM of each dNTP. All samples were subjected to amplification in a DNA thermal cycler 480 (Perkin-Elmer) with a step programme of 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. The amplified GRP78/BiP cDNA was separated on a 0.8% agarose-TBE gel containing ethidium bromide, purified from the agarose gel using the QIAEX gel extraction kit (Qiagen, Mississauga, ON) and ligated into T-ended pBluescript (KS) (Stratagene, La Jolla, CA). The ligation mixture was then used to transform competent DH5 α cells (Gibco/BRL). Plasmid DNA was isolated from transformed cells using the QIAEX miniprep kit (Qiagen), digested with *Hind*III and *Xho*I, and the GRP78/BiP cDNA insert purified from agarose. The GRP78/BiP cDNA insert was ligated into the *Hind*III/*Xho*I site of the mammalian expression vector pcDNA3.1(+) (Invitrogen, Carlsbad, CA) to produce the

recombinant plasmid, pcDNA3.1(+)-GRP78/BiP. Authenticity of the GRP78/BiP cDNA sequence was confirmed by fluorescence-based double stranded DNA sequencing (MOBIX).

The paragraph beginning on page 38, line 2 has been amended as follows:

SEQ ID NO:[1]4

Human GRP78/BiP amino acid sequence

MKLSLVAAMLLLSAARAEEEDKKEDVGTVGIDLGTTYSCGVFKNGRVEIIAND
QGNRITPSYVAFTPEGERLIGDAAKNQLTSNPENTVFDKRLIGRTWN DPSVQQDIKF
LPFKVVEKKT KPYIQVDIGGGQT KTFAP E EISAMV LTKM KETA E AY LGKKVTHAVV
TVPAYF NDAQRQATKDAGTIAGL NVMRII NEPTAAAIA YGLDKREGEKNILVFDLGG
GTFDV SLLTIDNGVFEVVATNGDTHLGGEFDQ RVMEHFIKLYKKKT GKDVRKDNR
AVQKL RREVEKAKRALSSQHQARIEIESFYEGEDFSETL TRAKFEELNMDLFRSTMKP
VQKVLEDSDLKKS DIDEIVLVGGSTRIPKIQQLVKEFFNGKEPSRGINPDEAVAYGAA
VQAGVLSGDQDTGDLVLLDVCPLTLGIETVGGVMTKLIPRNTVVPTKKSQIFSTASD
NQPTVTIKVYEGERPLTKDNHLLGTFDLTGIPPA PRGVPQIEVT FEIDVNGILRVTAED
KGTGNKNK IITNDQNRLTPEEIERMVNDAEKFAEEDKKLKERIDTRNELESYAYSLK
NQIGDKEKLGGKLSSEDKETMEKAVEEKIEWLESHQDADIEDFKAKKKELE
EIVQPIISKLYGSAGPPPTGEEDTAEKDEL

The paragraph beginning on page 38, line 20 has been amended as follows:

SEQ ID NO:[2]5

Human GRP78/BiP mRNA sequence

1 ACTGGCTGGC AAGATGAAGC TCTCCCTGGT GGCCGCGATG CTGCTGCTGC TCAGCGCGGC
61 GCGGGCCGAG GAGGAGGACA AGAAGGAGGA CGTGGGCACG GTGGTCGGCA TCGACCTGGG
121 GACCACCTAC TCCTGCGTCG GCGTGTCAA GAACGGCCGC GTGGAGATCA TCGCCAACGA
181 TCAGGGCAAC CGCATCACCG CGTCTATGT CGCCTTC ACT CCTGAAGGGG AACGCTGTAT
241 TGGCGATGCC GCCAAGAAC AGCTCACCTC CAACCCCGAG AACACGGTCT TTGACGCCAA
301 GCGGCTCATC GGCGCACGT GGAATGACCC GTCTGTGCAG CAGGACATCA AGTTCTTGCC
361 GTTCAAGGTG GTTAAAAGA AACTAAACC ATACATTCAA GTTGATATTG GAGGTGGGCA
421 AACAAAGACA TTTGCTCCTG AAGAAATTTC TGCCATGGTT CTCACTAAA TGAAAGAAC
481 CGCTGAGGCT TATTGGGAA AGAAGGTTAC CCATGCAGTT GTTACTGTAC CAGCCTATTT
541 TAATGATGCC CAACGCCAAG CAACCAAAGA CGCTGGAAC TATTGCTGGCC TAAATGTTAT
601 GAGGATCATC AACGAGCCTA CGGCAGCTGC TATTGCTTAT GGCCTGGATA AGAGGGAGGG

661 GGAGAAGAAC ATCCTGGTGT TTGACCTGGG TGGCGGAACC TTCGATGTGT CTCTTCTCAC
721 CATTGACAAT GGTGTCTTCG AAGTTGTGGC CACTAATGGA GATACTCATC TGGGTGGAGA
781 AGACTTTGAC CAGCGTGTCA TGGAACACTT CATCAAACGT TACAAAAAGA AGACGGGCAA
841 AGATGTCAGG AAAGACAATA GAGCTGTGCA GAAACTCCGG CGCGAGGTAG AAAAGGCCAA
901 ACGGGCCCTG TCTTCTCAGC ATCAAGCAAG AATTGAAATT GAGTCCTTCT ATGAAGGAGA
961 AGACTTTTCT GAGACCCCTGA CTCGGGCCAA ATTTGAAGAG CTCAACATGG ATCTGTTCCG
1021 GTCTACTATG AAGCCCGTCC AGAAAGTGTGTT GGAAGATTCT GATTGAAAGA AGTCTGATAT
1081 TGATGAAATT GTTCTTGTG GTGGCTCGAC TCGAATTCCA AAGATTCAAGC AACTGGTTAA
1141 AGAGTTCTTC AATGGCAAGG AACCATCCCG TGGCATAAAC CCAGATGAAG CTGTAGCGTA
1201 TGGTGCTGCT GTCCAGGCTG GTGTGCTCTC TGGTGATCAA GATACAGGTG ACCTGGTACT
1261 GCTTGATGTA TGTCCCCTTA CACTTGGTAT TGAAACTGTG GGAGGTGTCA TGACCAAAC
1321 GATTCCAAGG AACACAGTGG TGCCCTACCAA GAAGTCTCAG ATCTTTCTA CAGCTTCTGA
1381 TAATCAACCA ACTGTTACAA TCAAGGTCTA TGAAGGTGAA AGACCCCTGA CAAAAGACAA
1441 TCATCTTCTG GGTACATTG ATCTGACTGG AATTCTCCT GCTCCTCGTG GGGTCCCACA
1501 GATTGAAGTC ACCTTGAGA TAGATGTGAA TGGTATTCTT CGAGTGACAG CTGAAGACAA
1561 GGGTACAGGG AACAAAAATA AGATCACAAT CACCAATGAC CAGAATGCC TGACACCTGA
1621 AGAAATCGAA AGGATGGTTA ATGATGCTGA GAAGTTGCT GAGGAAGACA AAAAGCTCAA
1681 GGAGCGCATT GATACTAGAA ATGAGTTGGA AAGCTATGCC TATTCTCTAA AGAATCAGAT
1741 TGGAGATAAA GAAAAGCTGG GAGGTAAACT TTCCTCTGAA GATAAGGAGA CCATGGAAAA
1801 AGCTGTAGAA GAAAAGATTG AATGGCTGGA AAGCCACCAA GATGCTGACA TTGAAGACTT
1861 CAAAGCTAAG AAGAAGGAAC TGGAAAGAAAT TGTTCACCA ATTATCAGCA AACTCTATGG
1921 AAGTGCAGGC CCTCCCCCAA CTGGTGAAGA GGATACAGCA GAAAAAGATG AGTTGTAGAC
1981 ACTGATCTGC TAGTGTGTA ATATTGT